



Global response patterns of terrestrial plant species to nitrogen addition

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Summary

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- Better understanding of the responses of terrestrial plant species under global nitrogen (N) enrichment is critical for projection of changes in structure, functioning, and service of terrestrial ecosystems.
- Here, a meta-analysis of data from 304 studies was carried out to reveal the general response patterns of terrestrial plant species to the addition of N.
- Across 456 terrestrial plant species included in the analysis, biomass and N concentration were increased by 53.6 and 28.5%, respectively, under N enrichment. However, the N responses were dependent upon plant functional types, with significantly greater biomass increases in herbaceous than in woody species. Stimulation of plant biomass by the addition of N was enhanced when other resources were improved. In addition, the N responses of terrestrial plants decreased with increasing latitude and increased with annual precipitation.
- Dependence of the N responses of terrestrial plants on biological realms, functional types, tissues, other resources, and climatic factors revealed in this study can help to explain changes in species composition, diversity, community structure and ecosystem functioning under global N enrichment. These findings are critical in improving model simulation and projection of terrestrial carbon sequestration and its feedbacks to global climate change, especially when progressive N limitation is taken into consideration.

Key words: biological realm, biomass, functional type, latitude, nitrogen, plant species, precipitation, temperature.

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Introduction

Element nutrient has long been identified as one of the abiotic factors constraining plant growth, ever since Liebig's 'law of the minimum', which describes crop production as being limited by the nutrient in the shortest supply. Among the mineral nutrient elements, nitrogen (N) is recognized as the most widely limiting nutrient for plant growth both on land and in the sea (Vitousek & Howarth, 1991). Therefore, N fertilization is widely used to improve soil N availability and increase plant growth and productivity (Frink *et al.*, 1999). With regard to the unprecedented global climate change revealed by the four assessment reports of Intergovernmental Panel of Climate Change (IPCC) since 1990, it is of great concern whether the terrestrial biosphere acts as a net C sink

or source, that is, whether it poses negative or positive feedback to climate change. The third assessment report of the IPCC predicted 260–450 Pg C accumulation in terrestrial ecosystems under atmospheric CO₂ and climate change, 16–34% of the expected anthropogenic CO₂ emissions in an intermediate emissions scenario (Cramer *et al.*, 2001; IPCC, 2001). Based on the theory of ecological stoichiometry (Serner & Elser, 2002), the CO₂-climate projections on C accumulation require 2.3–16.9 Pg N, which may not be met under natural conditions (Hungate *et al.*, 2003). Elevated atmospheric CO₂ concentration can increase N immobilization by long-lived plant biomass and soil organic matter by stimulating plant growth, reducing N release and return to soil from litter decomposition, and decreasing N fixation over time (Hungate *et al.*, 2004). Consequently, soil N availability

will gradually decline and progressively constrain plant growth and net primary production (NPP; Luo *et al.*, 2004; Reich *et al.*, 2006), leading to lower C sequestration in terrestrial ecosystems than previously predicted. Thus, the central question of carbon–climate interactions is how N availability impacts the capacity of the terrestrial ecosystem to sequester C from the atmosphere (Gruber & Galloway, 2008; Heimann & Reichstein, 2008). Therefore, better understanding of how and to what extent N restrains terrestrial plant growth and NPP (Elser *et al.*, 2007; LeBauer & Treseder, 2008) is critical for a convincing projection of terrestrial C sequestration.

Along with global climate change, the global N cycle has also been profoundly altered by anthropogenic activities (Vitousek *et al.* 1997; Gruber & Galloway, 2008). Global N enrichment has been widely found to cause changes in community structure, reductions in species richness, and losses of biodiversity in various terrestrial biomes (Wedin & Tilman, 1996; Robbink *et al.*, 1998; Gough *et al.*, 2000; Zavaleta *et al.*, 2003; Stevens *et al.*, 2004; Suding *et al.*, 2005). Given the positive relationship of biodiversity and ecosystem stability (Tilman *et al.*, 2006), declines in plant diversity under N deposition/addition may result in greater variability (lower stability in reverse) in ecosystem function and services under environmental perturbations. Moreover, changes in community structure and biodiversity imply differential responses of growth of terrestrial plant species and their competitive ability under N addition. Therefore, understanding how terrestrial plant species respond to N addition can help to explain the changes in biodiversity, structure and functioning in terrestrial ecosystems.

There are more than 300 000 plant species across the world (Millennium Ecosystem Assessment, 2005), which can be categorized into different biological realms (e.g. seed plant, spore plant) and various functional types (e.g. growth forms, life history, and photosynthetic pathways). These categories differ in their N use strategy and grow in highly variable habitats depending on latitude and climate. Both habitats and plant functional types can impact plant responses to N addition and/or global change (Shaver & Chapin, 1980; Reich *et al.*, 2001, 2003, 2004; Van Wijk *et al.*, 2003). Regional and global patterns of tissue N concentrations of terrestrial plants have been widely reported along geographic and climatic gradients (Güsewell, 2004; McGroddy *et al.*, 2004; Reich & Oleksyn, 2004; Wright *et al.*, 2004; He *et al.*, 2006; Lovelock *et al.*, 2007). Moreover, N fertilization has long been conducted for a wide variety of plant species and terrestrial ecosystems (Frink *et al.*, 1999; Reich *et al.*, 2003). However, we still lack general response patterns of terrestrial plants at the species level to N addition (but, for NPP response, see Gough *et al.*, 2000; Elser *et al.*, 2007; LeBauer & Treseder, 2008), thus impeding the simulation and projection of climate–terrestrial C cycling feedback loops as well as changes in terrestrial ecosystem structure and functioning.

To reveal general response patterns of plant biomass and N concentration, we conducted a mixed-model meta-analysis using data from 456 terrestrial plant species (not including agricultural or horticultural, see Supplementary material, Tables S1–S3) in 304 published papers (see Text S1 and S2). In analyzing the response patterns of plant biomass and tissue N concentration to N addition, we incorporated factors such as geographic regions (latitude), climate (precipitation and temperature) gradients, biological realms (seed plant vs spore plant), growth forms (woody vs herbaceous species, grass, forb, shrub, and tree), life histories (annual herb vs perennial herb), photosynthetic pathways (C_3 herb vs C_4 herb) and other functional types (broadleaved tree vs coniferous tree, deciduous tree vs evergreen tree, legume vs nonlegume).

Materials and Methods

Data collection

We extracted results for plant biomass and tissue N concentration from N addition studies after building a database by searching the Science Citation Index (SCI) of the Institute of Scientific Information. References cited in a large number of N review articles and books were cross-checked to ensure inclusion of pre-1987 articles, which are not listed in the SCI. Any article published before June 2007 that met the following criteria was included in our analysis: reported responses of biomass and/or N concentration at the species level; species reported in the studies were those occurring in natural terrestrial ecosystems; means, standard deviations or standard errors with sampling sizes for both control and N addition treatments were provided. Articles that reported results on whole plants and parts of plants (e.g. leaf, shoot, root) were also included in our database. For the biomass analyses, different methods of biomass estimation (e.g. direct harvest for most herbaceous species and use of allometric relationships for some tree species) were accepted because we did not consider this to be a significant source of error in this analysis. Tree species at different ages were accepted. On the basis of these criteria, articles that reported responses to N addition at community level (Elser *et al.*, 2007; LeBauer & Treseder, 2008) and agricultural and horticultural species were excluded from our analysis. In addition, results from other proxy variables were not included in our analysis. For example, plant height or size was not included in biomass analysis, and tissue N content (e.g. g per plant) was not used in the analysis of N concentration. For biomass, the preferred metric was biomass per unit area (g m^{-2}), and other biomass data (e.g. g per plant) were transformed if information on plot area was provided in the paper. Otherwise, these data (e.g. g per plant) were also included in our analysis. Nitrogen concentration was transformed as N percentage (%) and used for analysis. Data with means and standard deviations for both control and N addition groups provided in the original

articles were used directly. Data (means and some measures of variance) presented in graphs were extracted by digitizing the figures using SigmaScan (Systat Software Inc., San Jose, CA, USA).

In meta-analyses, independence of the data being synthesized is assumed, and including multiple results from a single study violates this assumption, leading to alterations in the structure of the data, inflating samples and significance levels for statistical tests (Wolf, 1986; Vander Werf, 1992). Therefore some researchers have advocated the inclusion of only one result from each study (Vander Werf, 1992; Koricheva *et al.*, 1998; Liao *et al.*, 2008) when considering the lack of independence to be a serious problem for meta-analysis. However, the loss of information caused by the omission of multiple results in each study may become a more serious problem than that caused by violating the assumption of independence (Hedges & Olkin, 1985; Gurevitch *et al.*, 1992). Thus, many researchers have included more than one result from a single study in their meta-analyses (Gurevitch *et al.*, 1992; Wooster, 1994; Curtis, 1996; Curtis & Wang, 1998; Maestre *et al.*, 2005; Wang, 2007). Even though we made efforts to exclude duplicate results in different publications (e.g. some results published as figures in one paper and as tables in another), a large number of comparisons were used in our analysis because individual papers usually provided data from more than one treatment (e.g. varying N concentrations), different plant tissues (e.g. leaf, root), and/or different growing stages. Therefore, our estimates are not wholly independent. However, in order to minimize the degree of nonindependence in our study, we first averaged those data obtained in the same year for the same species under identical N treatment. Then, we conducted the analyses between biological realms and functional types again and found those patterns were unchanged (data not shown) when compared with the results using all data. On the other hand, we compared responses and sample sizes of different tissue types (leaf, root, branch/twigs, and wood) across woody species, in which distribution of tissue types could be more complex than herbaceous species. Although the uneven distributions of tissue types within categories could result in statistical biases, the proportions of each tissue type did not change substantially between categories (Table S4). Thus, all the results reported in our study were obtained by analyzing the data of all growing stages and tissue types.

We divided these data into two datasets: dataset 1 included only N addition without other resources (Text S1), and dataset 2 contained studies on N addition together with other resources (Text S2). In studies in dataset 2, if an effect of additional resources (e.g. CO₂ enrichment) on plants was given, we took this effect as the control, and the combined treatment (e.g. CO₂ enrichment plus N addition) as the N addition treatment. For dataset 1, because various parts of plants were included, we first roughly divided all data into above-ground tissue, below-ground tissue, and whole plants. We then

compared responses between above-ground and below-ground groups. We found that the responses among functional types were not changed except for N concentration among growth forms (Fig. S1). We also detected that responses between above- and below-ground tissues were similar within a plant functional type. For example, biomass responses were greater for above- than for below-ground tissues in most plant functional types, whereas N concentration responses showed no differences between above- and below-ground groups in most functional types (Fig. S1). On the other hand, sample sizes of below-ground tissues were much smaller than above-ground tissues in all functional types. Thus, all the three categories (above-ground tissues, below-ground tissues, and whole plants) of plant biomass and N concentration were included in our database to increase the number of plant categories.

There is substantial variability (from 1 g m⁻² in Lowe *et al.* (2003) to 100 g m⁻² in Loveland & Ungar (1983)) in the amount of N added among different studies. In order to test whether the amount of N added has an impact on plant responses, we first roughly partitioned added N into low (< 10 g N m⁻²) and high amounts (> 10 g N m⁻²) with similar sample size (Table S5). In addition, the large sample size of some plant functional types (herbaceous species, woody species, grass, forb, tree, and shrub) allowed us to further divide N amount into the following classes: < 5, 5–10, 10–15, 15–20, 20–25, and > 25 g N m⁻². For dataset 2, we categorized the N addition treatments into control (added N alone), facilitated (added N together with other resources), and limited (added N but limited by other abiotic factors). Because of the large sample size, we further tested the responses of added N together with phosphorus (P) addition and CO₂ enrichment.

We also collected other background information relevant to the data from the papers, including latitude, temperature and precipitation. For those studies with no geographical and climate information, we used data from the study sites where the experiments were conducted. Treatments that contained adding water or changing temperature were not included in the analysis of the impact of climate on plant N responses. For this type of analysis, we divided this background information into the following classes – latitude: 0–10, 10–20, 20–30, 30–40, 40–50, 50–60, and 60–70°; mean annual temperature (MAT): < 0, 0–5, 5–10, 10–15, 15–20, 20–25, and > 25°C; and mean annual precipitation (MAP): < 300, 300–600, 600–900, 900–1200, 1200–1500, and > 1500 mm.

Meta-analysis

The meta-analysis followed the techniques described in Wan *et al.* (2001). We used the natural log-transformed ratio ($\log_e r$) of plant biomass and tissue N concentration at elevated (X_e) to ambient (X_c) N to estimate the effect size of N treatment. A mixed-effects model was used in this analysis with the assumption that there are random variations in effect sizes among the diverse studies included in this synthesis.

Table 1 Between-group heterogeneity (Q_b) of nitrogen (N) effect size of biomass and N concentration across different biological realms and functional types

	Categories	Biomass		N concentration	
		Q_b	P	Q_b	P
Biological realms	Seed plant, spore plant	7.3	< 0.01	34.2	< 0.001
Growth forms	Woody, herbaceous	9.6	< 0.01	7.0	< 0.01
	Grass, forb, shrub, tree	95.8	< 0.001	39.7	< 0.001
Life history	Annual herb, perennial herb	13.2	< 0.001	5.5	< 0.05
Photosynthesis pathways	C ₃ herb, C ₄ herb	1.0	0.32	0.4	0.55
Other functional types	Broadleaved, coniferous tree	15.5	< 0.001	13.2	< 0.001
	Deciduous, evergreen tree	0.0	0.90	1.0	0.31
	Legume, nonlegume	12.3	< 0.001	51.3	< 0.001

Each individual observation is weighted by the reciprocal of the mixed-model variance (Curtis & Wang, 1998). The total heterogeneity of effect sizes among studies (Q_T) is partitioned into within-group (Q_w) and between-group (Q_b) heterogeneity. The Q statistic approximately follows a χ^2 distribution, which allows a significance test of the null hypothesis that all response ratios are equal. If Q_b is larger than a critical value, an independent variable has a significant influence on the response ratio (Gurevitch & Hedges, 1993). Confidence interval (CI) on effect-size estimates was generated by bootstrapping the data using MetaWin 2.1 (Sinauer Associates Inc., Sunderland, MA, USA). Nitrogen addition was considered to have a significant effect on a variable if the bootstrap CI of its percentage changes did not overlap zero. Responses of plant species were considered to significantly differ if their bootstrap CIs did not overlap (Gurevitch & Hedges, 1993). Statistical significance was tested at the $P < 0.05$ level.

Results

Dependence of N responses of terrestrial plants on biological realms and functional types

Across all the terrestrial plant species included in the present study, N addition increased biomass and N concentration by 53.6 and 28.5%, respectively. However, plants responded differently between the biological realms (Table 1, Fig. 1). N addition stimulated more biomass in seed plants (54.7%) than in spore plants (21.4%). However, a greater increase in N concentration in spore plants (49.4%) than in seed plants (26.8%) was observed in response to N addition (Fig. 1).

Nitrogen responses of terrestrial plants also varied with growth forms. Biomass responses of herbaceous species (62.3%) were greater than those of woody species (47.2%; Fig. 1a). By contrast, N addition increased tissue N concentration more in woody (29.2%) than in herbaceous species (23.9%; Table 1, Fig. 1b). N addition-induced enhancement of biomass in trees (61.0%) and grasses (78.7%) was much higher than that in shrubs (19.3%) and forbs (32.1%; Fig. 1a).

Positive responses of N concentration in forbs (13.6%) were significantly lower than those in grasses (30.1%), shrubs (29.8%), and trees (29.0%; Table 1, Fig. 1b).

For tree species, broadleaved trees responded more positively than coniferous trees in term of both biomass (73.1 vs 37.5%) and N concentration (34.6 vs 24.0%). However, no differences in the responses of either biomass or tissue N concentration were found between evergreen and deciduous trees (Fig. 1b). For herbaceous species, N addition caused greater increases in N concentration in annual (31.6%) than in perennial herbs (21.9%), and consequently led to much higher biomass stimulation for annual (92.4%) than perennial (56.1%) herbs. Nevertheless, responses of C₃ herbs did not differ from C₄ herbs in either biomass or N concentration (Table 1 and Fig. 1). It is expected that the competitive ability of N-fixing species will decrease under N addition compared with nonN-fixing species. Consistent with this proposition is the fact that growth stimulation of legumes by N addition was significantly lower than that of nonlegumes in both biomass (23.9 vs 46.5%) and tissue N concentration (6.3 vs 28.4%; Table 1, Fig. 1).

N responses of above- and below-ground plant tissues varied with functional groups

Between-group (plant functional types) N response patterns of above-ground tissues were similar to those of below-ground tissues (Fig. S1). When comparing within each group, biomass responses to N addition in seed plants were significantly greater in above- (56.1%) than in below-ground tissue (35.5%), but no differences in the responses of N concentration were found between above- and below-ground tissues (Fig. 2). Most functional types showed lower biomass responses in below- than in above-ground tissues. Response of above-ground biomass (47.9%) was significantly higher than that of below-ground biomass (23.0%) in woody species, while no difference was observed in herbaceous species. For woody species, there was a greater increase in above- than in below-ground biomass in both shrubs and trees. For tree species, a

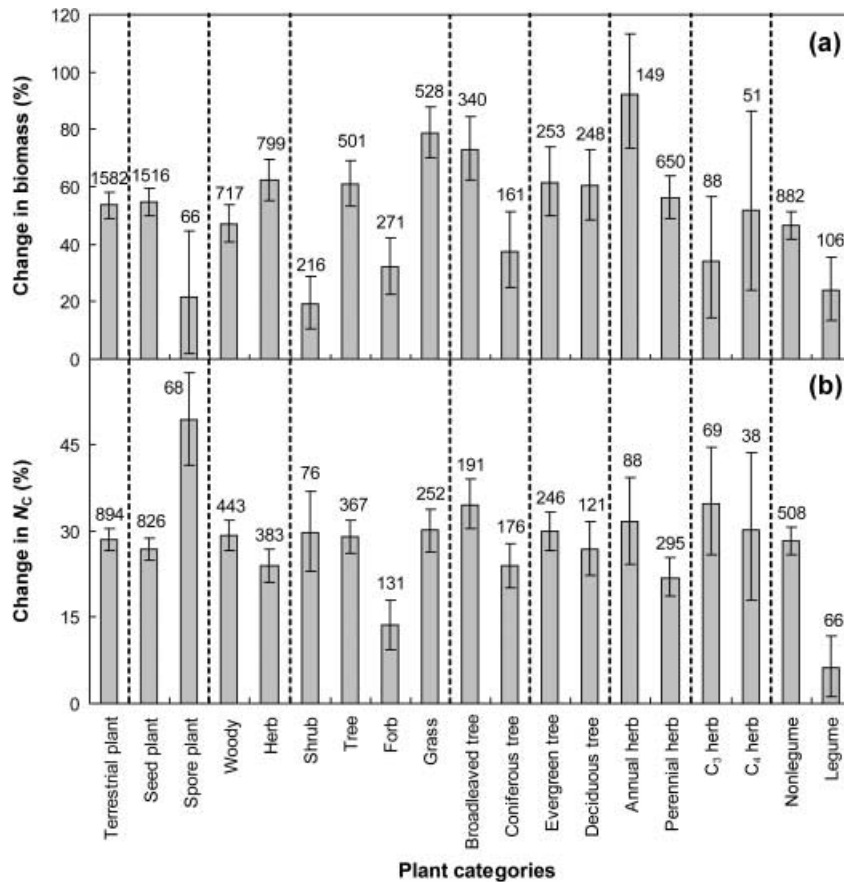


Fig. 1 Percentage changes in biomass (a) and N concentration (N_c) (b) for biological realms and all functional types included in this synthesis. Mean \pm 95% confidence interval. The number of observations for each category used in the analysis is shown near the bar.

greater response of above-ground biomass (83.4%) than of below-ground biomass (39.7%) was found in broadleaved trees only. For herbaceous species, above-ground biomass response was slightly, but insignificantly, higher than below-ground biomass response in forbs and perennial herbs, whereas the reverse was true for grasses and annual herbs. In addition, stimulation of above-ground growth (48.9%) in nonlegumes was significantly greater than that of below-ground tissue (22.1%), whereas legumes displayed similar increases in above- and below-ground biomass under N addition (Fig. 2a). Moreover, biomass responses in woody species to N addition varied with tissue type. Even though N addition stimulated growth for leaf, root, branch/twig and wood, biomass increases in branch/twigs (72.8%) and wood (50.5%) were significantly greater than those in both leaf (14.7%) and root (15.6%; Fig. 3a).

Responses of N concentrations were statistically insignificant between above- and below-ground tissues for both woody and herbaceous species. When separated into shrubs and trees, shrubs showed slightly, but insignificantly, higher increases in N concentrations in above- than in below-ground tissues, whereas trees showed significantly lower response in above- (25.8%) than in below-ground tissues (40.0%). Irrespective of statistical insignificance, below-ground responses of N

concentration were higher in all categories of trees (broadleaved trees, coniferous trees, evergreen trees, and deciduous trees). No difference in N concentration responses was found between above- and below-ground tissues for herbaceous species and nonlegumes. For legumes, N concentration of above-ground tissues (8.1%) was significantly increased by N addition. By contrast, no response of N concentration in below-ground tissues was observed (Fig. 2b). In addition, responses of N concentration in woody species varied with tissue type. In contrast to the biomass responses, there were greater increases in N concentration in leaf (29.2%), root (27.6%), and branch/twigs (24.9%) than in wood (11.4%), despite the fact that a significant difference was only observed between leaf and wood (Fig. 3b).

Variation in plant responses with amount of added N

There were no changes in the patterns of biomass response among plant functional types if we divided the amount of N into low ($< 10 \text{ g m}^{-2}$) and high ($> 10 \text{ g m}^{-2}$) values, except for herbs vs woody species and legumes vs nonlegumes (Table S5). Herbaceous species showed a significantly greater response (50.7%) than woody species (24.6%, $P < 0.01$) at low N supply, but no difference between these two functional types

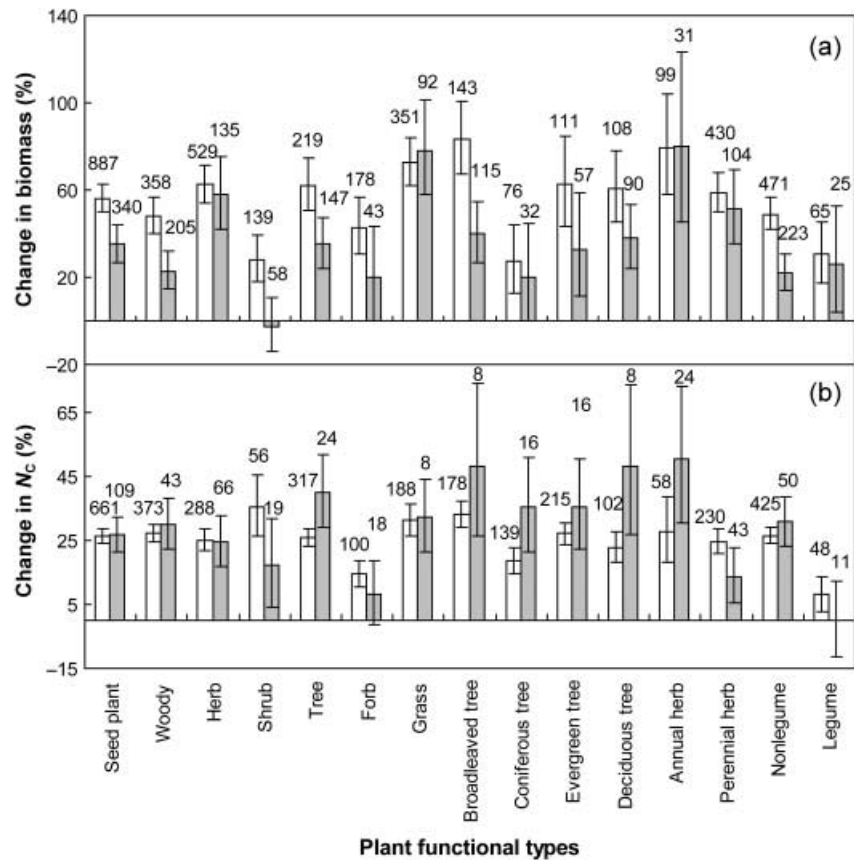


Fig. 2 Comparisons of above- (open bars) and below-ground (closed bars) responses to nitrogen (N) addition within plant functional types in biomass (a) and N concentration (N_c) (b). Mean \pm 95% confidence interval. The number of observations for each category used in the analysis is shown near the bar.

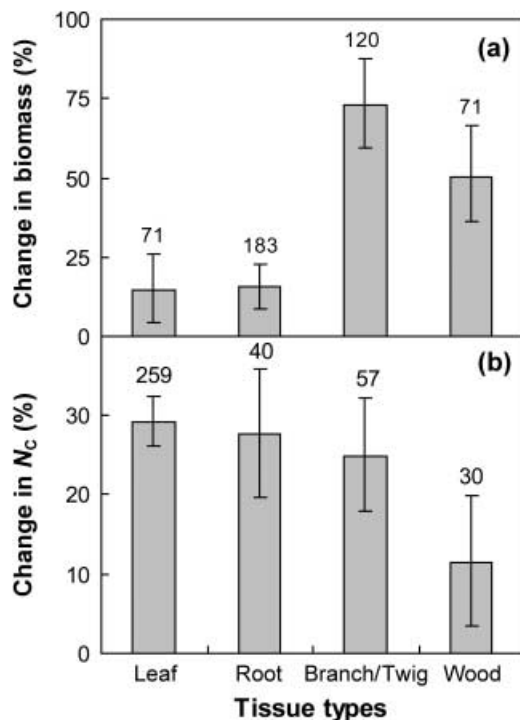


Fig. 3 Percentage changes in biomass (a) and N concentration (N_c) (b) for different tissue types of woody species. Mean \pm 95% confidence interval. The number of observations for each category used in the analysis is shown near the bar.

was found at high N supply. Consistent with the overall pattern between legumes and nonlegumes, both legumes (18.5%) and nonlegumes (23.4%) were stimulated by addition of low amounts of N. High amounts of N increased biomass of nonlegumes (43.3%), but did not affect legume growth.

When plotted along the N addition gradient, biomass responses of herbaceous species reached a peak when the added N was between 15 and 20 g m⁻² (Fig. 4a). Furthermore, woody species showed an increasing trend of biomass stimulation along the gradient of added N (Fig. 4a). For woody species, responses of trees tended to increase with amount of N, with the exception of 15–20 g N m⁻². Because of the sample size, we could not test the response of shrubs at 15–20 and 20–25 g N m⁻². However, growth of shrubs was only stimulated under the addition of < 10 g N m⁻², and there were no responses to the addition of 10–15 and > 25 g N m⁻² (Fig. 4b). For herbaceous species, all amounts of supplied N stimulated grass growth, whereas only amounts of between 10 and 20 g N m⁻² showed a significantly positive effect on forb growth (Fig. 4c).

Impacts of other resources

Growth of terrestrial plants is under the influence of multiple abiotic factors in addition to N availability. Irrespective of statistical insignificance, we detected relative differences in the

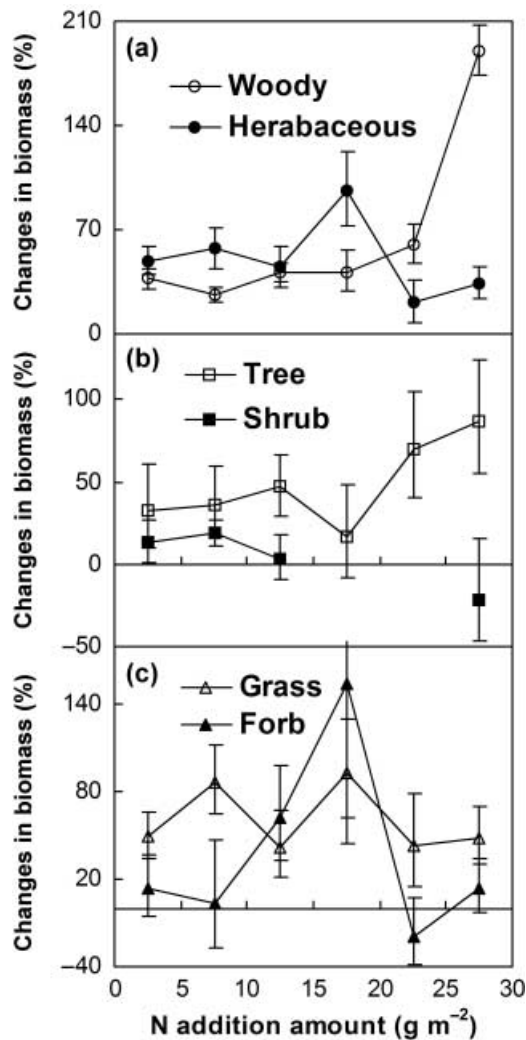


Fig. 4 Percentage changes in biomass for woody (trees and shrubs) and herbaceous (forbs and grasses) species under different amounts of nitrogen (N) addition (a–c). See effect size and sample size in Table S6.

response of plant biomass to N addition (Fig. 5), with facilitated conditions (63.5%) > control conditions (49.4%) > limited conditions (40.7%). The magnitudes of increase in N concentration also showed a declining trend from facilitated (33.9%) to control (31.6%) and limited conditions (28.6%). In those facilitated conditions, the effects of N addition on biomass were slightly promoted (24.9 vs 35.1%), but N addition suppressed N concentration (28.9 vs 24.3%) when P was added. CO₂ enrichment significantly enhanced the positive N responses of plant biomass from 68.3 to 104.2%, but no effect on N concentration (44.2 vs 38.3%) was found. If we divided the biomass responses to N addition under CO₂ enrichment into different functional types, we also found variation in the N responses among these functional types (Fig. 6). For example, N-induced biomass stimulation

of woody species (130.5%) was significantly greater than that of herbaceous species (71.3%) under CO₂ enrichment. For tree species, broadleaved trees showed a greater increase in biomass (166.3%) than coniferous trees (51.2%) under N addition. Biomass enhancement of deciduous trees (178.7%) was higher than that of evergreen trees (63.6%) in response to N addition.

Impacts of climatic conditions

Climatic factors, such as temperature and precipitation, can also have an impact on plant growth and its response to N addition. Increasing latitude from the equator to the poles represents a declining trend of MAT. Given the limitation of low temperature on plant activities, it is reasonable to predict that the N response at high latitudes would be lower than that at low latitudes. Our analysis revealed that biomass response of plant species to N addition logarithmically decreased with increasing latitude ($r^2 = 0.65$, $P < 0.05$, Fig. 7a). When plotted against MAT, the biomass responses of terrestrial plants to N addition could be divided into two groups (i.e. plants growing below and above MAT of 15°C). Plant species grown in MAT > 15°C exhibited greater biomass stimulation than those grown in MAT < 15°C (Fig. 7b). Across all the terrestrial plant species included in this study, changes in plant biomass increased linearly with MAP ($r^2 = 0.66$, $P < 0.05$, Fig. 7c).

Discussion

Various responses of biological realms and plant functional types to N addition

The average biomass response (53.6%) in our analysis is comparable to the result of a former meta-analysis of N addition studies across seven North American herbaceous ecosystems, where above-ground NPP increased by 50% under N fertilization (Gough *et al.*, 2000). The observations described in the Results section were much higher than those reported (approx. 28–29% stimulation of NPP) in two recent meta-analyses in terrestrial ecosystems across the globe (Fig. 1 in Elser *et al.*, 2007; LeBauer & Treseder, 2008). Irrespective of the general enhancement of biomass and tissue N concentration at the species level, our analyses clearly demonstrate that changes in biomass and tissue N concentration of terrestrial plant species in response to N addition are closely dependent on biological realms, plant functional types, and plant tissues.

The majority of studies on plant responses to N addition have focused on higher plants, especially seed plants (Fig. 1). Because of limited data on spore plants (e.g. moss, lichen, and fern), it is difficult to generalize their responses to N addition. However, we did observe variation in plant responses to N addition with biological realms, that is, greater stimulation of biomass and smaller increase in N concentration in seed

Fig. 5 Percentage changes in biomass (a, c) and nitrogen (N_c) concentration (b, d) in response to N addition under added N alone (control for a, b; N for c, d), added N but limited by other resources (limited), added N together with other resources (facilitated), added N together with P (NP), and added N together with elevated CO_2 concentration (NCO_2). The number of observations for each category used in the analysis is shown near the bar.

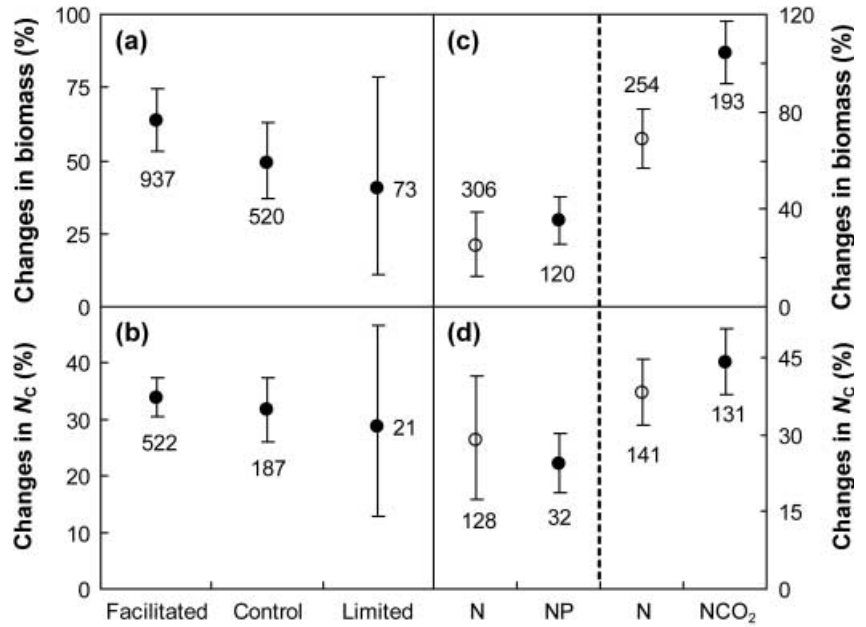


Fig. 6 Percentage changes in biomass for different functional types under nitrogen (N) addition in studies that added N together with elevated CO_2 concentration. Mean \pm 95% confidence interval. The number of observations for each category used in the analysis is shown near the bar.

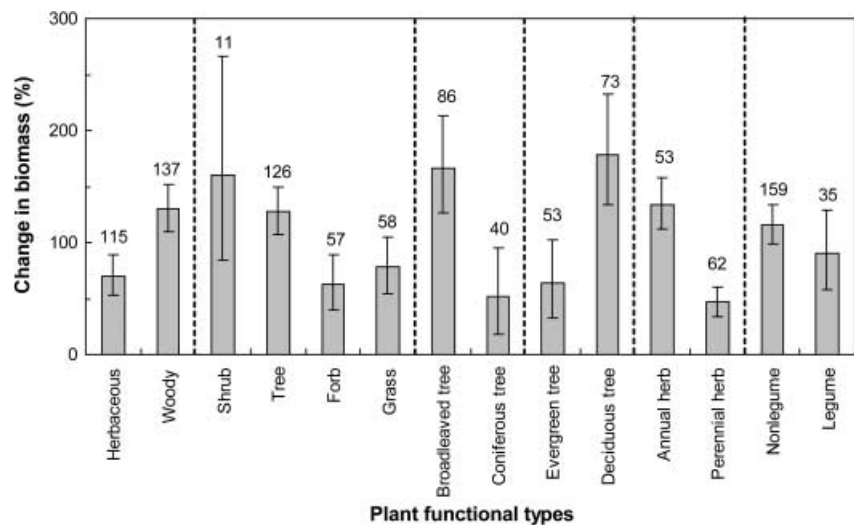
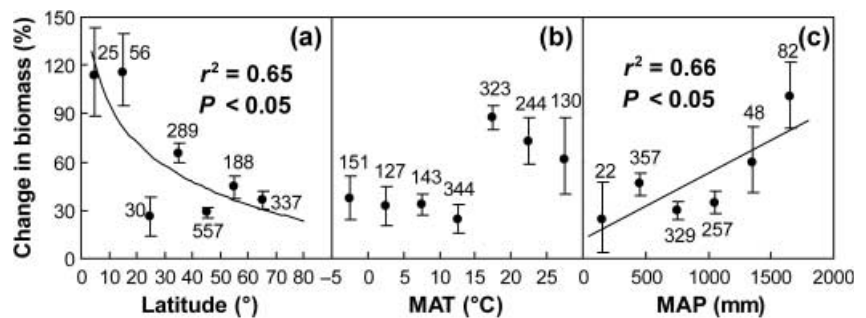


Fig. 7 Relationships between percentage changes in biomass under nitrogen (N) addition and latitude (a), mean annual temperature (MAT) (b), and mean annual precipitation (MAP) (c).



plants than in spore plants (Fig. 1). Nevertheless, because of the limited number of case studies and sample size in spore plants in comparison with the seed plants, we are not able to draw a solid conclusion about how N addition will affect the relationship between seed and spore plants, community structure and ecosystem functioning in natural terrestrial ecosystems where they coexist.

The differential responses among growth forms suggest that N enrichment has the potential to change plant species composition, community structure, and biodiversity in terrestrial ecosystems (Wedin & Tilman, 1996; Robbink *et al.*, 1998; Gough *et al.*, 2000; Zavaleta *et al.*, 2003; Stevens *et al.*, 2004; Suding *et al.*, 2005). Our finding of greater biomass response in herbaceous than in woody species is inconsistent with those observed at the ecosystem level, in which NPP is enhanced more in forests and shrublands than in grasslands (Fig. 2 in Elser *et al.*, 2007). However, our results showed that growth responses of herbaceous species are enhanced more than woody species at relatively low N supply ($< 20 \text{ g N m}^{-2}$), whereas biomass increases in woody species are greater than those of herbaceous species at high N supply (Fig. 4). This result indicates that herbaceous species need less N than woody species, possibly owing to their smaller stature, dry biomass, and N demand as well as faster N turnover via litter decomposition and release. For herbaceous species, grass growth was stimulated by all of the N supply levels, while increases in forb growth occurred exclusively at the median levels, suggesting that grasses, as a whole, are well adapted to stronger fluctuations in soil N availability than are forbs. For woody species, our findings that N addition stimulated greater biomass in trees than in shrubs are inconsistent with the observed findings in a former meta-analysis focusing on tropical ecosystems, in which shrubs respond more strongly than trees in respect of biomass accumulation (Lawrence, 2003). Tree growth was stimulated by almost all amounts of N supplied, while biomass of shrub (Fig. 4) was only increased by low amounts of N, indicating that tree growth is more sensitive to N supply than that of shrubs.

Differences in life history for herbaceous species can affect their N use strategy and responses to changes in N supply. In comparison with perennial herbs that maximize resource conservation, annual herbs have greater resource (e.g. N) acquisition (Chapin, 1980). As a consequence, annual herbs usually have higher N concentrations and lower N use efficiency than perennial herbs under similar N supply conditions. Therefore, annual herbs are likely to show greater biomass stimulation than perennial herbs under improved soil N availability (Fig. 1a). It has been predicted that N addition favors C_3 species more than C_4 species because of the lower N use efficiency of C_3 species (Wedin & Tilman, 1996; Sage & Kubien, 2003). However, in this analysis, there were insignificant differences between C_3 and C_4 species. The greater responses of nonlegumes than legumes are in line with the prediction that legumes will lose their advantage over other

nonlegume species if N availability is increased (Suding *et al.*, 2005). For woody species, our results showing no difference between the responses of deciduous and evergreen trees are inconsistent with the prediction that evergreen trees have more efficient at absorbing and utilizing mineral nutrient resources (Chapin, 1980) than deciduous trees. The greater responses of broadleaved than coniferous trees suggest an advantage of broadleaved over coniferous trees under N addition. The different responses between those covarying groups (e.g. broadleaved vs deciduous trees, coniferous vs evergreen trees) could be caused by the various responses in the subcategories, which include evergreen broadleaved trees (+91.5%), deciduous broadleaved trees (+61.5%), evergreen coniferous trees (+37.7%), and deciduous coniferous trees (+51.9%).

N responses of terrestrial plants vary with tissues

Greater stimulation of above-ground than below-ground growth in seed plants suggests amelioration of below-ground resource limitation and changes in above- and below-ground resource allocation under N addition. Greater resource allocation to above-ground growth indicates that N addition could shift below-ground competition for nutrients to above-ground competition for light resources (Tilman, 1987; Suding *et al.*, 2005). These results suggest that N addition may increase the risk of local extinction for those species that are able to tolerate low below-ground amounts of N (e.g. perennial herb, C_4 herb, and legumes) (Chapin, 1980; Craine *et al.*, 2002; Suding *et al.*, 2005).

Different tissues and organs function differently in woody species. As absorbing tissues for resources (water, nutrient, and carbon), roots (especially fine roots) and leaves are metabolically more active than branches and wood (trunk) and need more N for physiological activities. Therefore, N addition leads to greater increases in N concentration in roots and leaves than in branches and wood (Fig. 3b). Enhanced plant C uptake as a consequence of improved soil N availability and tissue N concentration results in stimulation of plant growth, which is mainly reflected by increased biomass accumulation in branches and wood (Fig. 3a) that function as structure, storage, and supporting organs (Chapin *et al.*, 2002).

Impacts of other resources on N effect

Based on Liebig's 'law of the minimum', many ecosystems in which biological processes were once limited by N are now limited more by other resources under N addition (Vitousek *et al.*, 1997). Our analysis demonstrates that the positive response of plants to N addition can be enhanced or suppressed when other resources are provided or limited, suggesting mediation of the growth responses of terrestrial plant species to N addition by other resources (water, P, K, CO_2 , etc.). The augmented positive N effect under addition of P observed in this study is consistent with the observations

at the ecosystem level reported by Elser *et al.* (2007) that simulation of NPP in response to N addition is significantly enhanced by P fertilization. The greater responses of plant biomass to N addition together with CO₂ enrichment suggest that the capacity of C sequestration in the terrestrial biosphere under elevated atmospheric CO₂ concentration can be enhanced by sustainable N supply (Reich *et al.*, 2006). The significantly greater biomass stimulation of woody species (130.1%) induced by N addition than herbaceous species (70.3%) under elevated CO₂ (Fig. 6) indicates that woody vegetation could sequester more C than herbaceous vegetation under elevated CO₂ as long as soil N availability can meet the requirements for plant uptake. By contrast, progressive N limitation (Luo *et al.* 2004) is more likely to occur in woody than in herbaceous vegetation if soil N supply is not sustainable for plant uptake.

Influences of geographical and climatic factors on plant N responses

Given its impact on numerous biological processes, temperature can influence plant growth and its responses to N addition. We observed a logarithmically declining trend of plant responses to N addition along the latitude gradient from the equator to the poles representing temperature gradients (Fig. 7a), suggesting constraints of low rather than high temperature on plant growth and its N response across the globe. Our result is inconsistent with a recent meta-analysis (LeBauer & Treseder, 2008), which found no correlation between N response of ANPP and latitude across all studies. In addition, given that N addition usually tends to favor species with lower N use efficiency (Wedin & Tilman, 1996), changes in leaf N concentration (the reverse is N use efficiency) along geographic gradients (McGroddy *et al.*, 2004; Reich & Oleksyn, 2004; Wright *et al.*, 2004; Lovelock *et al.*, 2007) can also impact the geographical patterns of plant response to N addition. Linear increases in N concentrations in green mangrove leaf tissue with increasing latitude from 4 to 37° (Lovelock *et al.*, 2007) imply lower N use efficiency and thus greater biomass responses at high-latitude regions. However, our analysis showed a declining trend of biomass stimulation of terrestrial plants along the latitudinal gradient, suggesting that temperature plays a more important role than plant N traits in regulating biomass stimulation under N addition along the latitudinal gradient.

However, the obvious division into two groups of the N responses of terrestrial plants (lower at low temperature and higher at high temperature) by a temperature threshold of 15°C (Fig. 7b) suggests some confounding factors may influence the effect of temperature on the growth responses of terrestrial plants to N addition. Similarly, MAT explains very little of the total variation in leaf N concentration of plants (Reich & Oleksyn, 2004). In a recent meta-analysis (LeBauer & Treseder, 2008), the greatest responses of forest NPP to N

addition occurred between 15 and 20°C, irrespective of the linear increase in the N effect with MAT. Although geographic distribution of plant species could contribute to the patterns under different MAT (e.g. herbaceous vs woody species or evergreen vs deciduous species; Reich & Oleksyn, 2004), there was no distinct difference in species distribution between 10–15 and 15–20°C in our analysis (data not shown). Thus, the underlying mechanisms for the step increases in the N responses of terrestrial plants around MAT of 15°C remain to be elucidated in the future.

The linear increases in the responses of plant biomass to N addition along the precipitation gradient (Fig. 7c) suggest that growth of terrestrial plants is co-limited by N and water availability. Water availability can be more important than N availability in regulating plant growth in regions with low annual precipitation, where the positive responses of plant growth to N addition are likely to be suppressed by water limitation. When water availability is no longer a limiting factor for plant growth under high annual precipitation, terrestrial plants often exhibit greater stimulation of biomass production in response to improved soil N availability. The increasing trend of the N responses of terrestrial plants with mean annual precipitation across the globe demonstrated in our meta-analysis is inconsistent with those findings by Hooper & Johnson (1999), who found that there is no increase in the relative response of above-ground net primary productivity (ANPP) to N with increasing precipitation based on several fertilization experiments in arid, semi-arid, and subhumid ecosystems.

Conclusions

The intensive alteration of global N cycles associated with anthropogenic activities (Vitousek *et al.*, 1997; Gruber & Galloway, 2008) has the potential to profoundly change plant growth and NPP in terrestrial ecosystems, with consequent impacts on global C cycles and climate change. Although it is difficult to extrapolate our species-level responses to terrestrial ecosystems directly, our results and previous findings (Elser *et al.*, 2007; LeBauer & Treseder, 2008) indicate that N limitation for plant growth is widespread at both the species and ecosystem levels in terrestrial biomes (Vitousek & Howarth, 1991). In addition, our observations of substantial variation in the plant responses to N addition with biological realms, plant functional types, plant tissues, other resources, and climate factors are critical for understanding the changes in plant species composition, diversity, community structure, and ecosystem functioning in terrestrial biomes. The findings in this and previous data syntheses (Elser *et al.*, 2007; LeBauer & Treseder, 2008) will facilitate model simulation and projection of terrestrial C sequestration and its feedbacks to global climate change, especially when progressive N limitation is taken into consideration (Hungate *et al.*, 2003; Luo *et al.*, 2004; Reich *et al.*, 2006).

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Supplementary Material

The following supplementary material is available for this article:

Fig. S1 Two figures showing between-group N responses for above- and below-ground tissues.

Text S1 List of studies used for analyzing effects of N addition treatment only in this meta-analysis.

Text S2 List of studies used for analyzing impacts of other resources on the N responses in this meta-analysis.

Table S1 List of plant species used for biomass in this meta-analysis

Table S2 List of plant species used for N concentration in this meta-analysis.

Table S3 Raw data in Excel format used in the meta-analysis.

Table S4 Distribution of sample size for different tissue types in woody species in this meta-analysis

Table S5 Weighted biomass response ratios (RR₊₊) and number of observations for all plant categories at low and high amounts of N

Table S6 Weighted response ratios (RR₊₊) and number of observations in biomass for some functional types (herbaceous species, woody species, grass, forb, tree, and shrub) under different amounts of added N

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